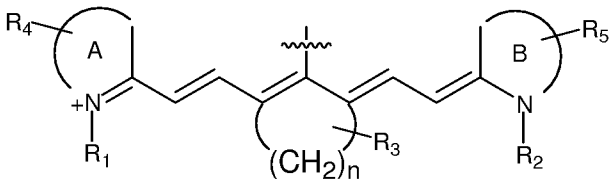
Specification Amendments

Please delete the paragraph beginning on page 2, line 4, and ending on page 2, line 19, and replace it with the following paragraph:

According to the present invention, fluorescent reporter compounds represented by the formula Z—L—Cy are provided. According to this embodiment, Z is a nucleotide; L is a linker of sufficient length to connect the nucleotide derivative to the cyanine dye, such that the cyanine dye does not significantly interfere with the overall binding and recognition of the nucleotide derivative by a nucleic acid replication enzyme, such as diradical moiety having a chain length of at least 8 atoms; and Cy is a cyanine dye of the formula:



wherein

A and B are each independently the atoms necessary to form a cyanine nucleus ~~nuclei~~;

R_1 and R_2 are each independently C_1-C_6 alkyl;

R_3 is hydrogen, or C_1-C_4 alkyl;

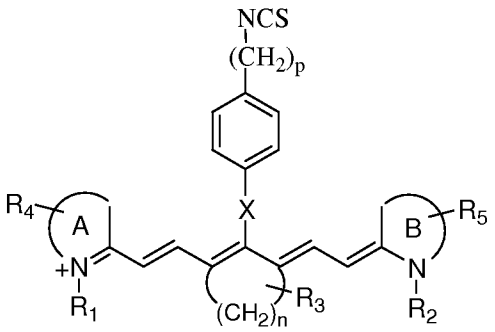
R_4 and R_5 are each independently selected from the group consisting of H and SO_3^-; and

n is an integer between and including 2-4.

Please delete the paragraph beginning on page 3, line 5, and ending on page 3, line 7, and replace it with the following paragraph:

It is preferable, but not required that the fluorescent reporter compounds according to the present invention have a fluorescence ~~maxima~~ maximum greater than 750 nm, as measured in an aqueous solution.

Please delete the paragraphs beginning on page 4, line 1, and ending on page 5, line 2, and replace it with the following paragraphs:



wherein

A and B are each independently the atoms necessary to form a cyanine nucleus ~~nuclei~~;

X is O, S, NR_9, or CR_9R_{10};

R_1 and R_2 are each independently C_1-C_6 alkyl;

R_3 is hydrogen, or C_1-C_4 alkyl;

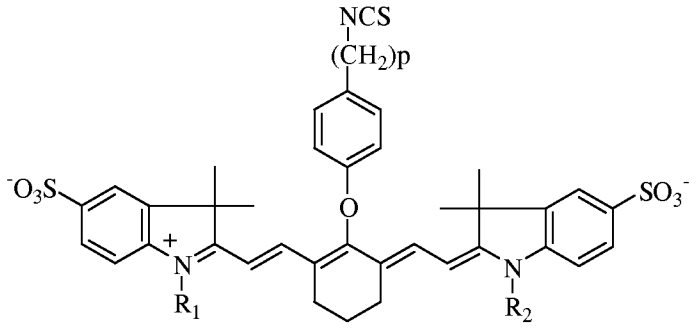
R_4 and R_5 are each independently selected from the group consisting of H and SO_3^-, provided that at least one of R_4 and R_5 is SO_3^-;

R_9 and R_{10} are each independently H or C_1-C_4 alkyl;

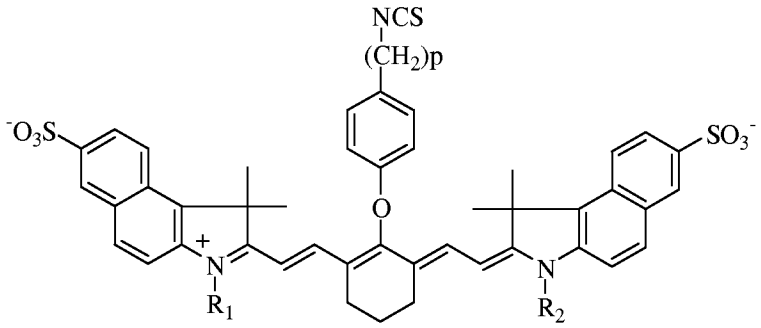
p is an integer between and including 2-8; and

n is an integer between and including 2-4.

In the above described cyanine dyes, it is preferable, but not required, that R_4 and R_5 are both SO_3^-, and R_1 and R_2 are both C_1-C_4 alkyl. More preferably, R_1 and R_2 are identical. Examples of preferred, but not required, cyanine dyes include compounds of the following formulas:



and



Please delete the paragraph beginning on page 6, line 23, and ending on page 7, line 5, and replace it with the following paragraph:

The cyanine dyes according to the present invention are coupled to a nucleotide derivative through the ring-locked portion of the cyanine chromophore, as shown in Formula 1 above, through a linker. Ring-locking the cyanine dye chromophore and attaching the nucleotide derivative through the ring-locked portion of the cyanine dye provides fluorescent labeled reporter compounds with enhanced stability, solubility, and quantum yield. These compounds have absorbance and emission frequencies in the near infrared region, a large extinction coefficient, and solubility in aqueous solutions. In addition, the fluorescent labeled reporter ~~reported~~ compounds can be incorporated into a nucleic acid chain termination reaction in high yield.

Please delete the paragraph beginning on page 7, line 7, and ending on page 7, line 12, and replace it with the following paragraph:

The term "cyanine nucleus ~~nuclei~~" means the carbon, hydrogen, and hetero-atoms necessary to complete the conjugated system that makes up a fluorescent cyanine chromophore. Cyanine nuclei that can be used in the fluorescent labels according to the present invention are known to those skilled in the art. Examples of cyanine nuclei include substituted or unsubstituted thiazole, benzothiazole, napthothiazole, benzoxazole, napthoxazole, benzolselanazole, napthoselenazole, indole, and benzoindole rings.

Please delete the paragraph beginning on page 7, line 21, and ending on page 7, line 24, and replace it with the following paragraph:

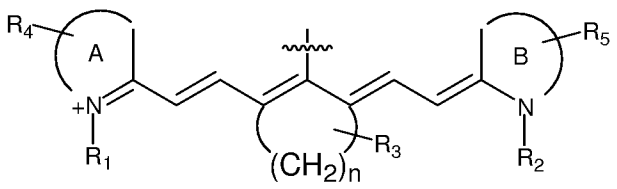
As used in this disclosure, the term "phosphate functionality" means a mono-, di-, or tri-phosphate, or a phosphate analog such as an alpha-thiotriphosphate ~~alpha-thiotripposphate~~, that when joined to a nucleoside derivative forms a nucleotide derivative that is capable of being used by a replication enzyme to attach the nucleotide derivative to a nucleic acid sequence.

Please delete the paragraph beginning on page 8, line 3, and ending on page 8, line 6, and replace it with the following paragraph:

In one embodiment, the present invention is a fluorescent labeled reporter compound having a modified cyanine dye attached to a nucleotide derivative. A preferred, but not required, ring-locked cyanine fluorescent labeled reporter compound according to the present invention is shown in Formula 2.

Please delete the paragraph beginning on page 8, line 21, and ending on page 9, line 8, and replace it with the following paragraph:

Also shown in Formula 2 above is "Cy" which represents a cyanine dye of the formula:



wherein

A and B are each independently the atoms necessary to form a cyanine

nucleus ~~nuclei~~;

R_1 and R_2 are each independently C_1-C_6 alkyl;

R_3 is hydrogen, or C_1-C_4 alkyl;

R_4 and R_5 are each independently selected from the group consisting of H and SO_3^-; and

n is an integer between and including 2-4.

Please delete the paragraph beginning on page 11, line 7, and ending on page 11, line 8, and replace it with the following paragraph:

The heterocyclic-base is the portion of the nucleotide derivative that functions as the recognition element in nucleotide synthesis. Generally, these are a purine or pyrimidine base that correspond to a natural nucleic acid ~~base~~. Examples of heterocyclic-bases including ~~cytosine~~ ~~cytocine~~, deazaadenine, deazaguanine, deazahypoxanthine, and uracil are shown below.

Please delete the paragraphs beginning on page 13, line 4, and ending on page 14, line 10, and replace it with the following paragraphs:

As shown in Scheme 1, ~~an~~ iminium salts (**1A**) and (**1B**), which represent the cyanine nuclei, are reacted with a bis-amino cycloalkene (**2**) to form the cyanine chromophore (**3**). Cyanine (**3**) is then derivatized by substituting the leaving group (LG) on the ring-locked portion of the cyanine chromophore with a phenyl ethylisothiocyanate compound (**4**) to form the cyanine dye (**5**). The nucleotide derivative "Z" is then coupled to the cyanine dye (**5**) by coupling the pendant alkynyl amine on nucleotide derivative (**6**) with the isothiocyanate portion of cyanine dye (**5**) to form the fluorescent reporter compound (**7**).

In a preferred, but not required, embodiment, modified cyanine dyes having an indole ring structure in the cyanine chromophore, according to the present invention are synthesized as shown below in Scheme 2. As shown in Scheme 2, first, a hydrazinobenzene (**8**) is reacted with a ketone, to form the indole ring **(9)**. The amine in the indole ring is then coupled with an alkyl halide to form an iminium salt (**10**). Two equivalents of the iminium salt (**10**) are then reacted with a bis-amino chloro-cyclohexene compound (**11**) to produce the cyanine dye (**12**). Cyanine dye (**12**) is then further derivatized by substituting the chlorine on the ring-locked portion of the cyanine dye with the (hydroxyphenyl)ethylisothiocyanate compound (**13**) to form the cyanine dye (**14**).

Please delete the paragraph beginning on page 14, line 28, and ending on page 15, line 8, and replace it with the following paragraph:

In another preferred, but not required, embodiment, modified cyanine dyes having a benzoindole ring structure in the cyanine chromophore, according to the present invention, are synthesized as shown below in Scheme 3. As shown in Scheme 3, first, benzoindole (**15**) is sulfonated to form the benzoindole (**16**). The benzoindole (**16**) is then coupled with an alkyl halide to form an iminium salt (**17**). Two equivalents of the iminium salt (**17**) are then reacted with the bis-amino chloro-cyclohexene compound (**11**) to produce the cyanine dye (**18**). Cyanine dye (**18**) is then further derivatized by substituting the chlorine on the ring-locked portion of the cyanine dye with the (hydroxyphenyl)ethylisothiocyanate compound (**13**) to form the cyanine dye (**19**).

Please delete the paragraphs beginning on page 24, line 12, and ending on page 25, line 1, and replace it with the following paragraphs:

Referring now to Figure 1A, the normalized absorbance spectrum ~~spectra~~ of cyanine dye **14**, Ring-locked Cy7, was compared the normalized absorbance spectrum ~~spectra~~ of known cyanine dye Cy7 in gel buffer and in water.

Referring now to Figure 1B, the normalized fluorescence spectrum ~~spectra~~ of cyanine dye **14**, Ring-locked Cy7, was compared the normalized fluorescence spectrum ~~spectra~~ of known cyanine dye Cy7 in gel buffer and in water.

Referring now to Figure 2A, the normalized absorbance spectrum ~~spectra~~ of cyanine dye **19**, Ring-locked DBCy7, was compared the normalized absorbance spectrum ~~spectra~~ of known cyanine dye DBCy7 in gel buffer and in water.

Referring now to Figure 2B, the normalized fluorescence spectrum ~~spectra~~ of cyanine dye **19**, Ring-locked DBCy7, was compared the normalized fluorescence spectrum ~~spectra~~ of known cyanine dye DBCy7 in gel buffer and in water.